

Seedling survival of *Handroanthus impetiginosus* (Mart ex DC) Mattos in a semi-arid environment through modified germination speed and post-germination desiccation tolerance

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Abstract

Uniform rapid seed germination generally forms a great risk for the plant population if subsequent intermittent precipitation causes desiccation and seedling death. *Handroanthus impetiginosus* can be found commonly in a wide range of biomes within Brazil including those that are semi-arid. Germination and early growth was studied to understand how germinated seeds survive under these stringent conditions. Accessions were sampled from four seasonally dry biomes in Brazil. Precipitation at the start of the rainy season in the Caatinga, a semi-arid biome, is less predictable and the number of successive dry days per dry interval in the first four months of the rainy season was higher than in the other studied biomes. Plants from the Caatinga produced thicker seeds and this trait concurred with slow germination and stronger osmotic inhibition of germination across the accessions, forming a stress avoidance mechanism in the Caatinga. Post-germination desiccation tolerance was high in the Caatinga accession, could be re-induced in accessions from biomes with more regular precipitation (Cerrado and transition zone), but remained poor in the Cerradão accession; thus forming a stress tolerance mechanism. Production of adventitious roots ascertained survival of all tested individuals from all four locations, even if protruded radicles did not survive desiccation, forming an additional stress tolerance mechanism. A sequence of stress avoidance and stress tolerance mechanisms in seeds and germinated seeds was associated with precipitation patterns in different biomes. These mechanisms purportedly allow rapid seedling establishment when conditions are suitable and enable survival of the young seedling when conditions are adverse.

Keywords: adaptation, germination, post-germination desiccation tolerance, seed shape, stress tolerance.

Sobrevivência de plântulas de *Handroanthus impetiginosus* (Mart ex DC) Mattos em ambiente semi-árido ocorre por modificação da velocidade de germinação e da tolerância à dessecação pós germinação

Resumo

A germinação rápida e uniforme geralmente pode apresentar riscos para a população de plantas caso a precipitação ocorra de maneira intermitente, provocando seca e morte das plântulas. *Handroanthus impetiginosus* pode ser encontrado em uma grande variedade de biomas no Brasil. A germinação e o crescimento inicial das plântulas de *Handroanthus impetiginosus* foram estudadas para compreender como as sementes germinadas sobrevivem nestas condições. Acessos de quatro biomas que apresentam secas sazonais foram amostrados. A precipitação, no início da estação chuvosa na Caatinga, um bioma semi-árido, é menos previsível e o número de dias secos consecutivos por intervalo de seca, nos primeiros quatro meses da estação chuvosa, foi maior do que nos outros biomas estudados. Plantas do bioma Caatinga produzem sementes mais espessas e essa característica está associada com uma germinação lenta e com a inibição

osmótica maior da germinação através dos acessos, formando um mecanismo para evitar o estresse. Tolerância à dessecação em sementes germinadas foi superior no bioma Caatinga e que podem ser reinduzida em acesso com uma precipitação mais regular (Cerrado e na zona de transição), mas manteve-se baixa no acesso Cerradão, formando assim um mecanismo para evitar o estresse. Produção de raízes adventícias foi observada nos indivíduos estudados de todos os quatro biomas, mesmo quando as raízes primárias não sobreviveram à dessecação, formando um mecanismo adicional de tolerância ao estresse. Assim, foi observado estratégias para evitar o estresse e mecanismos de tolerância ao estresse em sementes e sementes germinadas associada com padrões de precipitação nos diferentes biomas estudados. Estes mecanismos podem favorecer o estabelecimento das plântulas de forma rápida quando as condições são adequadas e permitirem a sobrevivência de plântulas quando as condições são adversas.

Palavras-chave: adaptação, germinação, tolerância à dessecação pós-germinação, formato da semente, tolerância ao estresse.

1. Introduction

Water is a universal requirement for life and the most abundant component in all active cells. For this reason, environmental water is a crucial resource for survival of organisms and determines the geographical distribution of the species on the planet (Oliver et al., 2005). Fluctuating availability of water in most regions has exerted evolutionary pressures that have provided plants to develop several strategies for survival in extremely dry environments; namely avoidance, resistance, or tolerance to desiccation, especially in reproductive structures such as spores, pollen and seeds (Levitt, 1972; Oliver et al., 2000; Leprince and Buitink, 2010). In species with wide geographical distribution it has been reported that different climatic conditions may produce seeds with different sizes, shapes and degrees of desiccation tolerance (Dussert et al., 2000; Tweddle et al., 2003; Pritchard et al., 2004). Desiccation tolerance acquired at the end of embryogenesis is naturally present in orthodox seeds. During seed germination and seedling development, the desiccation tolerance is lost progressively and varies between species; *Arabidopsis thaliana* seeds lose desiccation tolerance during germination, while *Medicago truncatula* Gaertn. loses desiccation tolerance after germination is completed (Kermode, 1995; Buitink et al., 2003; Maia et al., 2011). This makes the seedling development stages highly vulnerable to drought and associated with high mortality (Harper, 1977). However, several studies have shown the possibility of re-establishment of desiccation tolerance in germinated seeds when drying to less than 0.1 g H₂O g⁻¹ dw (Bruggink and van der Toorn, 1995; Leprince et al., 2000; Pukacka, 2001; Buitink et al., 2003; Faria et al., 2005; Vieira et al., 2010).

Handroanthus impetiginosus is an arboreal species with wind-dispersion and with extensive geographical distribution in the American tropics in wide range of biomes, from wetter biomes like Amazon and Atlantic Forest to drier biomes such as the Cerrado and Caatinga (Lorenzi, 2002; Schulze et al., 2008). *H. impetiginosus* seeds have not shown dormancy and germination is very rapid (Vieira et al., 2010), imposing a risk of mortality for the young seedlings particularly in the drier biomes.

Re-establishment of desiccation tolerance was reported in *H. impetiginosus* after osmotic treatment of germinated seeds with radicles up to 3 mm long. Furthermore, germinated

seeds with radicles that turned necrotic, displayed the ability to form adventitious roots from the hypocotyl. Both phenomena hypothetically form an adaptation to dry environmental conditions that occur in biomes such as the Cerrado and Caatinga, providing survival of the organism under selective pressure (Vieira et al., 2010). Accessions from wetter biomes are expected to be less tolerant to desiccation than those from drier biomes. Moreover, rapid germination has been associated with high-stress habitats, while many of these species have dimorphism with a different germination speed (Parsons, 2012). Therefore, it is conceivable that intraspecific variation in germination speed plays a role in the adaptation to environments with contrasting water availability, thus forming a stress avoidance mechanism.

We evaluated various seed traits in a range of accessions of *H. impetiginosus* from different regions within Brazil that are all characterised by limited water availability in the drier winter season, and where seed germination in the field normally takes place at the start of the rainy season. The occurrence of early rainfall followed by a dry spell imposes a risk of seedling death through desiccation. Biometry, reserve composition, germination, osmotic inhibition and the re-establishment of desiccation tolerance of germinated seeds were assessed. Water availability forms a strong driver in natural selection, and we used the data to test the hypothesis that seed traits are associated with precipitation patterns.

2. Material and Methods

2.1. Climate data

Multi-year data were obtained from the Instituto Nacional de Meteorologia (INMET), Departamento de Água e Esgoto de Penápolis (DAEP) and Empresa de Pesquisa e Agropecuária do Rio Grande do Norte (EMPARN). Data from five recent years with complete precipitation data (2001, 2004, 2005, 2006 and 2007) were used for analysis of seasonal precipitation patterns. Meteorological data showed that Açú rainy season occurs in a different period from the other localities. Thus, Within each year, data from August until November (Rondonópolis, Penápolis and Lavras) and from November to February (Açú) were used to describe the dry spells that separated the days with precipitation at the start of the rainy season.

2.2. Plant material

Seeds of *H. impetiginosus*, synonyms *Tabebuia impetiginosa* and *Tecoma impetiginosa*, were collected from a range of (sub-) biomes: Cerrado *sensu stricto*, Cerradão, Caatinga and transition zone between Atlantic Forest and Cerrado. Seeds from the Cerrado *sensu stricto* were collected near Rondonópolis in the state of Mato Grosso (16° 27' S, 54° 34' W) at an altitude of 227 meters with a climate classification Awh (Köppen, 1948). Seeds from the Cerradão were collected near Penápolis in the state of São Paulo (21° 25' S, 50° 04' W) at an altitude of 416 meters with a climate classification Cwa (Köppen, 1948). Seeds from the Caatinga biome were collected near Açú in the State of Rio Grande do Norte (5° 34' S, 36° 54' W) at an altitude of 23 meters with climate classification Bsh (Köppen, 1948). Seeds from a transition region between Atlantic forest and Cerrado were collected near Lavras in the state of Minas Gerais (21° 13' S, 44° 58' W) at an altitude of 918 meters with a climate classification Cwa (Köppen, 1948).

Mature fruits were collected between the last week of September and first week of October of 2007 from at least ten adult trees per location. Fruit and seed maturity were verified using morphological parameters (Carvalho et al., 2008). The seeds were extracted manually from the fruits, mixed, dried to a moisture content of 0.07 g H₂O g⁻¹ dw and stored for five months in plastic bags in a controlled environment room with 55% relative humidity and 5 °C. The moisture content of seeds was determined by weighing four replicates of 10 g of seeds before and after drying at 105 °C ± 3 °C for 24 hours. Integuments were removed before each experiment, producing seeds that consisted of an embryo enveloped by an endothelium (Souza et al., 2005).

2.3. Biometry

The length, width and thickness were measured in 30 seeds from each location. The mass determination was performed in ten replicates of 100 seeds from each region.

2.4. Stored reserves

The analyses of stored reserves were performed in four repetitions of 30 seeds each, upon grinding to a fine powder. The quantitative starch content was determined according to McCready et al. (1950) by using four replicates of two grams each. The reducing sugar content was extracted according to Cunniff (1995) and quantified according to Smogyi (1952), in four repetitions of five grams each. The total protein content was determined according to Sathe and Venkatachalam (2007) using the micro-Kjeldahl method described by Cunniff (1995), in four replicates of 0.1 g each. The lipids were extracted with ethyl ether in Soxhlet and quantified according to Cunniff (1995) using four replicates of two grams each.

2.5. Germination

Prior to the germination experiment the seeds were placed in a moist chamber at 100% RH for 24 hours. Subsequently, the seeds were surface-sterilised in 2%

sodium hypochlorite for 30 seconds and washed in ample distilled water for another 30 seconds. The seeds were placed to germinate in 8.5 cm Petri dishes on two layers of filter paper. Four replicates of 20 seeds each, for each location, were used in 5 mL of distilled water, polyethylene glycol 8000 (PEG) solution, or 100 µM of the ABA biosynthesis inhibitor fluridone (Sigma-Aldrich, St Louis, MO, USA; Li and Walton, 1990). Water potential of PEG solutions was calculated according to Michel (1983). Upon imbibition, seeds were incubated at 30 ± 1 °C in the presence of continuous light. Seeds were considered to have completed germination when the radicle protruded through the endothelium and its length exceeded 1 mm within seven days from the start of imbibition. The time until half-maximal germination (*t*₅₀) was calculated for each dish using a sigmoidal curve fit (Boltzmann equation; Origin 6.1); thus, high *t*₅₀ represented slow germination. The osmotic inhibition coefficient that characterised the stress tolerance of seeds was defined as the slope of the linear relationship between water potential of PEG solutions and the *t*₅₀ in these solutions.

2.6. Re-establishment of desiccation tolerance

The re-establishment of desiccation tolerance in germinated seeds of *H. impetiginosus*, or post-germination desiccation tolerance, was assessed by the ability of the protruded radicle to survive desiccation according to Vieira et al. (2010); with or without incubation in a PEG solution (-1.7 MPa at 5 °C for 72 h in the dark) prior to desiccation.

2.7. Statistical analysis

Normality of data was tested with a Shapiro-Wilk test. If data were not normally distributed Kruskal-Wallis ANOVA (precipitation and biometry) or a Friedman's test (stored reserves) was applied with paired comparisons, while a general ANOVA with Duncan's multiple range test was applied if data followed a normal distribution (the 100-seed mass and *t*₅₀). Osmotic inhibition coefficients for the geographical locations were compared by testing the slopes upon regression analysis of *t*₅₀ against osmotic potential. Spearman's rank correlation was applied to all parameters. All tests were performed in GenStat v14 (VSN International Ltd.).

3. Results

The four geographical locations where seeds were collected displayed different monthly precipitation patterns. For all four locations (Açú, Lavras, Rondonópolis and Penápolis) most precipitation occurred mainly in the summer season, while winters were characterised by long dry spells during which flowering and fruiting occurred. However, the dry season at the Northerly location Açú that is located in the Caatinga lasted longer (six months) and started around one month later than at the other three locations. Consequently, total annual precipitation in Açú (628 mm) was around half the amount of that in the other three locations, and accessions from Açú experienced drier

conditions than those from the other three geographical locations (as shown in Table 1). Mean precipitation was generally low in all four locations during the months of July and August when seeds developed and matured, but the lowest in Açú (16mm) and the highest in Penápolis located in the Cerradão (59 mm; as shown in Table 1). Not only was the annual and July/August precipitation lower in Açú: the occurrence of rainfall at the end of the dry season was lower and less regular, indicated by the duration of dry spells and the relatively higher variance for this parameter.

The 100seed mass differed almost two-fold between the four geographical locations, ranging from 5.68 g in Penápolis to 10.97 g in Lavras (as shown in Table 1); the seed length, width and thickness also differed. The 100seed mass was positively correlated with seed width ($P=0.037$, $R=0.926$). These results indicated that the thickest seeds with a relatively high mass were produced in Açú where precipitation during seed development was the lowest and where precipitation at the start of the rainy season was more intermittent. The content of stored reserves (starch, sugar, protein, and lipid) differed between the four locations (as shown in Table 1), but was not correlated with most other parameters. Only the precipitation during seed development in July and August was positively correlated with the starch content ($P=0.045$, $R=0.910$) but not with the content of reducing sugars, lipids and proteins.

In all accessions the final germination reached 95% or higher, and did not differ. The germination speed in water t_{50} differed between the four locations ($P<0.001$), with the slowest germination observed for the seeds from Açú. The seed thickness was correlated with the t_{50} ($P=0.018$, $R=0.965$; see Figure 1a). Fluridone, applied in a concentration of 100 μM , improved the germination speed in the accessions by 11-14 h. However, this improvement did not appear to differ between the four accessions (data not shown); indicating that endogenous abscisic acid did not play a role in the differences in t_{50} . The t_{50} declined linearly with a decreasing water potential ($P<0.001$) and differed between the four accessions ($P=0.004$, see Figure 1c). The osmotic inhibition coefficient, defined as the slope of the linear relationship between water potential and t_{50} , was the highest in Açú (131) and the lowest in Penápolis (88) and these osmotic inhibition coefficients differed significantly ($P=0.001$) but not from those of Lavras and Rondonópolis. A high osmotic inhibition coefficient was observed in thick seeds ($P=0.045$, $R=-0.910$; see Figure 1b) and was associated with a high t_{50} (slow germination; $P=0.058$, $R=-0.885$). The data suggested that thicker seeds display slower germination and stronger osmotic inhibition.

Post-germination desiccation tolerance was determined for germinated seeds of the four accessions subsequent to PEG treatment to improve the post-germination desiccation tolerance, or without such prior treatment. The accession from Açú displayed a high degree of post-germination desiccation tolerance in the control treatment (see Figure 2a). The accessions from Lavras and Rondonópolis had limited desiccation tolerance in the control treatment that was

lower than the desiccation tolerance of the accession from Açú ($P<0.001$); while the desiccation tolerance of the accession from Penápolis was the lowest and differed from that of Rondonópolis ($P=0.002$). PEG treatment improved the survival of the protruded radicles by 21% across the range of treatments and accessions ($P<0.001$; see Figure 2b). Post-germination desiccation tolerance

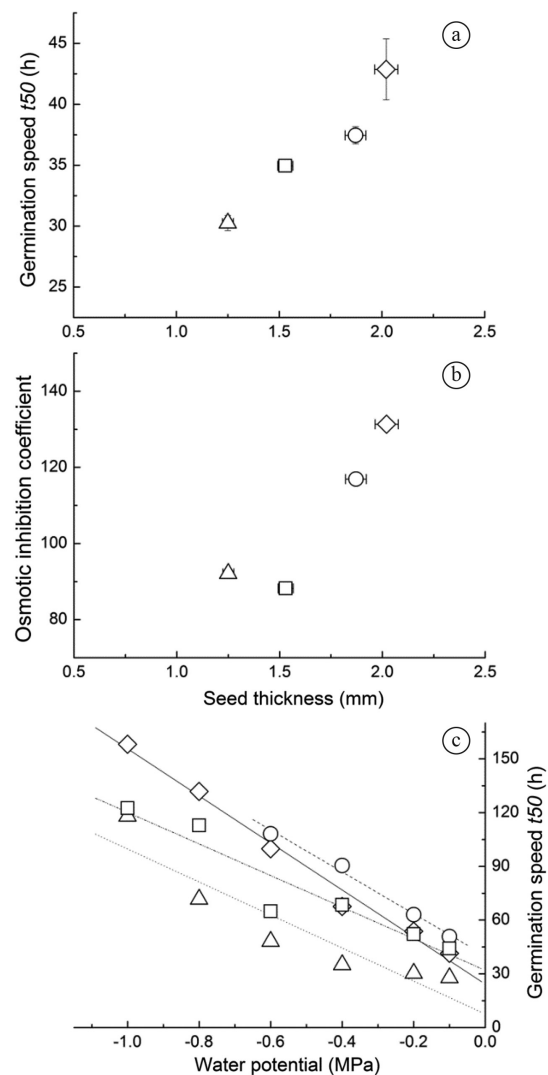


Figure 1. Seed thickness versus germination speed t_{50} (a) and osmotic inhibition coefficient (b) of the accessions from Açú (\diamond), Lavras (\circ), Rondonópolis (\triangle) and Penápolis (\square). Data are means and standard error; $n=5$ for osmotic inhibition coefficient, $n=4$ for t_{50} , $n=30$ for seed thickness. The results for Duncan's test for t_{50} were Açú > Lavras = Penápolis > Rondonópolis. Water potential of the imbibition medium versus t_{50} (c) of the four accessions from Açú (\diamond , solid line), Lavras (\circ , dashed line), Rondonópolis (\triangle , dotted line) and Penápolis (\square , dashed dotted line). Data are means, $n=4$; lines are linear curve fits. The slopes of the linear curve fits were 131 for Açú, 117 for Lavras, 92 for Rondonópolis and 88 for Penápolis.

Table 1. Annual precipitation, precipitation in July and August, and the number of successive dry days intermitted by days with precipitation at the start of the rainy season in the four geographical locations in Brazil; 100seed mass, seed length, width and thickness for the four accessions.

| Geographic origin | Annual rainfall (mm) | Rainfall Jul-Aug (mm) | Successive dry days (n) | 100seed mass (g) | Length (mm) | Width (mm) | Thickness (mm) | Starch (mg.g ⁻¹) | Reducing sugar (mg.g ⁻¹) | Protein (mg.g ⁻¹) | Lipid (mg.g ⁻¹) |
|-------------------|----------------------|-----------------------|-------------------------|------------------|--------------|-------------|----------------|------------------------------|--------------------------------------|-------------------------------|-----------------------------|
| Açú | 628 ± 92 b | 16 ± 8 b | 12.6 ± 3.5 a | 9.3 ± 0.2 b | 10.5 ± 0.1 b | 8.2 ± 0.1 b | 2.0 ± 0.1 a | 31 ± 0.7 b | 2.4 ± 0.1 a | 238 ± 0.5 b | 343 ± 13 b |
| Lavras | 1391 ± 45 a | 34 ± 9 b | 3.9 ± 0.9 bc | 11.0 ± 0.4 a | 13.0 ± 0.2 a | 9.3 ± 0.1 a | 1.9 ± 0.1 a | 36 ± 1.2 a | 1.8 ± 0.0 b | 206 ± 1.1 c | 395 ± 5 a |
| Rondonópolis | 1311 ± 68 a | 19 ± 8 ab | 3.6 ± 0.2 c | 7.5 ± 0.1 c | 10.2 ± 0.1 b | 8.3 ± 0.2 b | 1.3 ± 0.0 c | 30 ± 0.9 b | 1.5 ± 0.0 c | 255 ± 0.6 a | 318 ± 9 b |
| Penápolis | 1254 ± 63 a | 59 ± 15 a | 4.7 ± 0.5 ab | 5.7 ± 0.2 d | 9.7 ± 0.1 c | 7.4 ± 0.1 c | 1.5 ± 0.0 b | 38 ± 1.1 a | 1.9 ± 0.1 b | 241 ± 0.4 b | 351 ± 8 b |
| | P=0.006 | P=0.033 | P=0.048 | P<0.001 | P<0.001 | P<0.001 | P<0.001 | P=0.002 | P=0.008 | P<0.001 | P<0.001 |

Data are means ± s.e.; n=5 years for precipitation, n=10 for mass and n=30 for length, width and thickness. Data for content of stored seed reserves are means ± s.e.; n=4. Values followed by different letters in each column do not differ in a post-hoc comparison test.

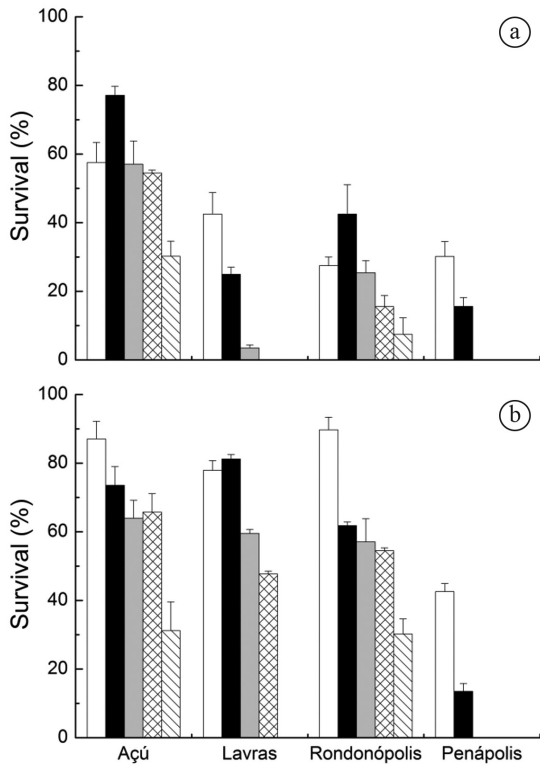


Figure 2. Survival of protruded radicles with a length of 1.5 (open bars), 2.0 (closed bars), 2.5 (grey bars), 3.0 (cross-hatched bars), or 3.5 mm (hatched bars) without (a) or with prior PEG treatment (b) of the accessions from Açú, Lavras, Rondonópolis and Penápolis. Data are means and standard error, $n=4$.

was not significantly improved by PEG treatment in the accession from Açú, but improved more than 3-fold in the accession from Lavras ($P<0.001$) and more than 2-fold in the accession from Rondonópolis ($P<0.001$). Furthermore, survival of these accessions no longer differed from the accession from Açú upon PEG treatment. For the accession from Penápolis desiccation tolerance remained low and no significant improvement through PEG-treatment was observed across the tested development stages. Across the range of treatments germinated seeds with longer protruded radicles demonstrated poorer survival than those with shorter protruded radicles, both for PEG-treated and control groups ($P<0.001$). Nevertheless, none of the germinated seeds of any of the four accessions died, whether or not being able to re-establish desiccation tolerance in the protruded radicle. In all of the germinated seeds adventitious roots emerged from the hypocotyls that ensured the survival of 100% of the seedlings, even if necrosis of the protruded radicle occurred (data not shown).

4. Discussion

Seed and seedling traits have been considered to crucially reflect the ecological and evolutionary life history of plants and they correlated with impediment of gene

flow and random selective pressures of the environment (Ackerly et al., 2000; Valladares et al., 2000; Moles and Leishman, 2008; Soriano et al., 2011). In the present study *H. impetiginosus* from different biomes exhibited considerable variation in ecophysiological traits. Seeds produced in an environment with drier winters tended to have a higher mass, whereas seeds produced in biomes with more and more regular winter precipitation had a smaller mass. The opposite relationship was described for seed mass and annual precipitation in 12,987 species (Moles et al., 2005). However, rather than the annual precipitation, the current data suggest that the seasonal precipitation pattern and the unpredictable rainfall at the start of the rainy season plays a role in the seed mass and size of *H. impetiginosus*. Seed thickness was correlated with the osmotic inhibition coefficient and $t50$ in water and the production of these thicker seeds in Açú suggests adaptation to the limited water availability in this semi-arid environment. Slower germination increases the chance of survival under environmental conditions with both long and erratic dry spells upon the first rainfall at the end of the dry season. Unpredictable precipitation is common in tropical dry forests (Vieira et al., 2008). Slow germination was associated with unpredictable rainfall in a range of neo-tropical pioneer species, to reduce the risk of high seedling mortality (Daws et al., 2007). Such unpredictability of the precipitation frequency formed a stress that was additional to the low annual precipitation (as shown in Table 1). Some desiccation studies have reported that a large size and rounded shape of reproductive structures can affect the surface area-to-volume ratio and the distance water must diffuse from the interior of the tissue to the surface, reducing the speed of drying (Pammenter et al., 2002; Pritchard et al., 2004). Conversely, the thicker seeds of Açú may be protected more from desiccation if initial rainfall is followed by a dry spell. Since thickness is the smallest of the seed dimensions an increase in thickness would have a relatively large effect on the spherical shape. Osmotic inhibition of germination was associated with seed thickness and was the strongest in the accession from Açú in the Caatinga, where precipitation was low and less predictable at the start of the rainy season. Osmotic inhibition acts as a mechanism to sense the limited availability of water in the environment, and offers the seed the possibility to delay the start of seedling growth that forms a vulnerable phase in establishment and development. This most likely occurs through equilibration of the seed to the low water potential of the drying soil, upon the first rain after dispersion. Osmotic inhibition of germination is a wide-spread phenomenon that has been demonstrated for various species including several crops (Bewley and Black, 1994). Both slow germination and strong osmotic inhibition contribute to delayed soil emergence and to stress avoidance, which is a more conserved strategy that offers adaptive value in an environment where precipitation is less predictable.

Lipids formed the major storage compound, followed by proteins, reducing sugars and starch. Although the

content of these storage compounds differed between the accessions no association with *t50* was observed. Seed protein content has been associated with germination behaviour (Brancalion et al., 2008). Modifying the lipid and protein content may contribute to the regulation of imbibition speed (Gardarin et al., 2011; Soriano et al., 2011). However, in *H. impetiginosus* this seemed to play no role; instead, the seed dimensions were associated with the speed of germination. Larger seeds produced larger initial seedlings in a range of neotropical trees that tended to survive better but grow more slowly (Baraloto et al., 2005). Such presumed slower growth of seedlings produced from larger seeds, in addition to the slower germination, may form an additional benefit in environments that are more arid and where precipitation is less predictable. However, this hypothesis requires further investigation. Differences in dormancy and germination have been associated with abscisic acid synthesis (Ali-Rachedi et al., 2004). Endogenous abscisic acid synthesis did not explain the differences in germination between the accessions, since applied fluridone reduced germination speed similarly across the accessions. The endothelium that covers the embryo is also unlikely to cause differences in the germination speed, since this tissue was very thin in all accessions.

Molecular aspects of the re-establishment of desiccation tolerance have been studied extensively in the model species *Medicago truncatula* (e.g. Buitink et al., 2003, 2006; Boudet et al., 2006) and *Arabidopsis* (Maia et al., 2011). However, although an ecological role for this phenomenon seems obvious, this has not been studied in-depth. *H. impetiginosus* displayed re-establishment of desiccation tolerance in germinated seeds that was naturally high in the accession from Açú; could be increased with osmotic treatment to high levels in the accessions from Lavras and Rondonópolis, while both the natural and inducible desiccation tolerance of the Penápolis accession was poor. The concurrence with the semi-arid climate and unpredictability of precipitation at the start of the rainy season indicates that the re-establishment of desiccation tolerance in germinated seeds can be considered an adaptation for an opportunistic species to limited water availability in order to minimize seedling mortality, yet providing a competitive advantage by allowing early germination and seedling establishment. Since this mechanism is likely to be energetically costly, it is conceivable that the trait is lost if not required when growing in an environment where precipitation is more abundant and predictable, as was observed in the Penápolis accession. The vegetation type of the Cerradão around Penápolis, which is a closed canopy forest unlike the other biomes, may facilitate the retention of water in the soil and seedling for this latter accession, which would explain the poor post-germination desiccation tolerance and the lack of inducible desiccation tolerance. Interestingly, all individuals from each of the four geographical locations displayed full regeneration and produced healthy seedlings by producing adventitious roots, once desiccation tolerance was lost and upon necrosis of the protruded radicles. This phenomenon was

explained as an adaptation to seasonally dry environments (Vieira et al., 2010). While this implies functional redundancy for post-germination desiccation tolerance of the radicle, there may be an additional advantage for this phenomenon. Strong winds causing damage and uprooting of young trees has been associated with larger taproots in pine trees (Moore et al., 2008). Therefore, survival of the protruded radicle that forms the taproot has an advantage over replacement by adventitious roots. *H. impetiginosus* was described as a Caatinga species with indistinct distribution patterns that include Amazonian localities (Prado and Gibbs, 1993), but as a species of the Neotropical Seasonally Dry Forests (SDF) by Collevatti et al. (2011). Most of South-America experienced a considerably drier climate between 22,000 and 11,000 years than at present, and expansion of the Neotropical Seasonally Dry Forests (SDF) into the Amazonia basin during the Pleistocene was proposed (Pennington et al., 2000). *H. impetiginosus* has been observed throughout most of the Amazon rainforest (Schulze et al., 2008); which may be the result of a persistent geographical presence in a climate that has changed since the most recent global glaciation event. These published data indicate that ancestral populations of *H. impetiginosus* are well-adapted to arid climates and that more recently in geological history populations were engulfed by vegetation types without seasonal drought. Furthermore, this implies that the trend for reduced desiccation tolerance and reduced stress avoidance is the result of adaptation to a wetter climate with higher predictability for precipitation.

In conclusion, *H. impetiginosus* displayed natural variation for a range of physiological traits, resulting in a sequence of development events in the seeds and seedlings that appeared to offer adaptation to the unpredictability of precipitation at the start of the rainy season, varying in four Brazilian biomes. Seed thickness was associated with the speed of germination, which together with osmotic inhibition controlled the timing of radicle protrusion; these events lead to stress avoidance for the seedlings in the Caatinga with less precipitation and lower predictability of precipitation. Post-germination desiccation tolerance of the protruded radicle and production of adventitious roots in the germinated seed acted subsequently during development to promote survival of the young seedling; these events formed part of a stress tolerance mechanism. These stress avoidance and stress tolerance traits likely serve a reduction in seedling mortality in a seasonally arid environment, while allowing rapid seedling development under favourable environmental conditions. Relaxation of these traits may have contributed to the widespread geographical distribution of the species in the tropical Americas and its current presence in a range of biomes with distinct climates and precipitation patterns.

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